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Cereal Leaf Beetle Survey and Biocontrol Activities in Washington State, 2002

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Background

The cereal leaf beetle, *Oulema melanopus* (L.), is an exotic leaf-feeding insect pest of small grains such as oats, wheat, and barley. Originally from Europe, the cereal leaf beetle (CLB) was first detected in the United States in Michigan in 1962 (Castro et al. 1965). Since then, the geographic range of CLB in the US has increased to include most eastern states and many western states such as Utah, Idaho, Montana, Wyoming, and Oregon (NAPIS 2001; Bai et al. 2002).

The first detection of cereal leaf beetle in Washington occurred in 1999 at four sites in Spokane County (Klaus et al.1999). Survey efforts were expanded in 2000 and 2001, resulting in detections in eight additional counties: Adams, Clark, Columbia, Franklin, Grant, Lincoln, Pend Oreille and Stevens (Hitchcox et al. 2000; 2001).

Entomologists from the Washington State Department of Agriculture (WSDA), the U.S. Department of Agriculture (USDA) and Washington State University (WSU) are working cooperatively to protect small grain growers in Washington State. An annual ***detection survey*** is designed to qualify the status of cereal leaf beetle in Washington counties, to facilitate early detection of infestations, and to satisfy external quarantine regulations. A ***delimitation survey*** is designed to quantify levels of infestation, and to monitor the growth and control of local populations over time. A ***biological control program*** has been implemented to suppress CLB infestations through the use of natural enemies. Selected natural enemies (parasitoids) are imported from western states to CLB-infested sites in Washington for propagation. Once established, the parasitoids disperse naturally or are redistributed to other infested fields, ultimately reducing the CLB populations to below economic threshold levels. This report presents the results of the 2002 CLB survey and biocontrol activities.

2002 Project Objectives

1. **Continue statewide detection survey, with efforts placed on "high priority" counties.**

2. **Continue delimiting the geographical distribution of, and continue monitoring the density of, known CLB populations.**

3. **Continue biological control efforts through the establishment and maintenance of field insectary sites, and the acquisition and release of selected biocontrol agents.**

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PROJECT METHODS

1. Detection Survey

Survey methods in 2002 were the same as those used in 2001 and were designed to adhere to guidelines and standards established by the western Cereal Leaf Beetle Working Group (CLBWG). The CLBWG serves as an informal scientific forum and regional partnership to aid in the exchange and redistribution of biological control agents. The group represents 10 western states and provinces. Its members include industry representatives, federal and state agricultural entomologists, and university researchers and extension agents.

In order to allocate sampling efforts, counties were classified as **low** or **high** priority based on three criteria:

1) *survey history:* number of years and intensity of sampling completed in previous years,
2) *pathway:* proximity to previous CLB detection sites, and likelihood of introduction, and
3) *host crop*: the number of acres of production of cultivated host crops, such as wheat, barley, oats, rye, triticale, and forage crops (grass hays, wild oats, etc.).

High-priority counties have no or little survey history, are located adjacent to an infested site and/or along major traffic routes, and possess a relatively high number of acres planted with host crops. The survey set a goal of 20 sites in high-priority counties, and a minimum of five sites in low-priority counties. If detections were made before the target number of sites was reached, the detection survey would be complete in that county. Any further survey in a positive county was conducted as a delimitation survey (see below).

Site selection favored lush green fields of commercial grain such as oats, wheat, barley and rye. In areas with little grain production, sampling included wild and cultivated grasses such as timothy, orchard grass, bluegrass, wild oats, corn, brome, and ryegrass and reed canary grass. The survey start date threshold occurred when average daily air temperatures were over 50°F for three consecutive days. The start date was determined by viewing daily temperature and wind data monitored by the WSU Public Agricultural Weather System (PAWS 2002).

Essentials of survey methods include both visual inspection and sweep netting. For survey purposes, a “sweep” was defined as one pass through the upper foliage with a 15” diameter sweep net. A “sample” was defined as 30 sweeps taken at a moderate walking pace 10-15 ft. inside the border of the field. Four samples were taken from each site, totaling 120 sweeps per site. The contents of each sample were visually inspected for life stages of CLB and all suspect specimens were retained for verification and reference. Visual inspection was conducted between sweeps by observing for CLB life stages and feeding damage on the host plants.

2. Delimitation Survey

A delimitation survey is conducted as a multi-year effort to monitor growth and movement of localized CLB populations in positive counties with agriculturally significant areas. The delimitation goals in 2002 included surveying regions of Adams, Columbia, Franklin, Grant, Lincoln, Spokane, and Stevens counties. The survey focused on areas in and around known infested fields. Special

attention was placed on potential avenues of natural and human-assisted dispersal such as river corridors, railway systems, and highways.

To monitor long-term population changes, estimates of population density were conducted at selected sites in Spokane, Clark, Grant and Lincoln counties. Estimates were determined at each site by performing a visual inspection of host plant leaves on 100 tillers. At each site, four separate sections of a field were selected, and 25 tillers per section were randomly sampled. Plant leaves were inspected and all CLB eggs and larvae were counted and recorded.

3. ***Biological Control***

Since 2000, a proactive biological control program has been conducted, aimed at long-term suppression of cereal leaf beetle populations in Washington State. With the cooperation of local farmers, field insectaries are established on commercial farms in areas where CLB infestations have been found. The field insectaries are managed to encourage the establishment and survival of selected bioagents and to reflect the management guidelines described by the USDA-PPQ Niles Biological Control Lab (USDA-Niles). In 2002, the CLB biocontrol program was fortified with the addition of a second field insectary site in Spokane County and the allocation of acreage at the Nine Mile Falls insectary site.

Parasitic wasp Tetrastichus julis attacking a CLB larva at the Nine Mile Falls insectary

The parasitic wasp species *Tetrastichus julis* is the only species of bioagent currently being introduced in Washington State. In 2002, Washington received shipments of the bioagent *T. julis* through cooperators in Montana* and Pennsylvania†. Parasitized CLB larvae were collected from field insectaries in Pennsylvania and Montana between June 13 and June 27. Cooperators collected CLB larvae either by hand or via sweep net. A subsample of CLB larvae was collected and analyzed by staff at the Niles Biological Control Lab to determine percent parasitism. All shipments of parasitized CLB larvae (bioagents) were released at the Nine Mile Falls field insectary by WSDA, USDA, and WSU staff. The bioagents were released directly into the field in designated conservation strips or field insectary plots.

Pre-release Survey

A “pre-release” study was conducted to monitor for naturally occurring larval parasitoids and to determine establishment of introduced bioagents from the previous years’ release. In June, prior to any 2002 releases, WSDA and WSU personnel conducted a pre-release survey at the Nine Mile Falls field insectary. CLB larvae were collected from “conservation strips” in cultivated wheat fields where earlier releases had been made. Collected larvae were stored in 20% ethyl alcohol at 45°F for no more than one week. The majority of larvae were sent directly to USDA-Niles for dissection and examination. A number of dissections were also completed by WSDA.

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RESULTS AND DISCUSSION

1. Detection Survey

The 2002 survey began on May1 and ended on August 14. Standardized detection survey was completed at 275 sites, with confirmed detections at five new sites. New detections were located in the counties of Asotin, Ferry, Garfield, Lewis, and Whitman (Table 1). At each positive site, initial sweep and visual inspection resulted in the capture of single CLB specimens. Follow-up sweeps and inspection produced no further specimens, except at the Ferry County site, where four additional larvae were found. Detections in Asotin, Ferry, Garfield and Lewis counties occurred during scheduled survey activities. In Whitman County, a single specimen was collected during a WSU entomology study at Lyle Grove Biological Area, a wooded area located eight miles southwest of Pullman. The adult specimen was found in samples from two malaise traps operated throughout the summer. Due to the late-season capture of this specimen, no follow-up survey was possible in 2002. Delimiting surveys are scheduled for 2003 and will attempt to locate any established populations.

Table 1: CLB Detection Survey Results, 2002

County	Target # Sites	# Sites Sampled	# New Positive Sites	# Specimens Recovered
Asotin	5	3	**1**	1 adult
Benton	20	13	0	
Chelan	5	2	0	
Clallam	5	5	0	
Cowlitz	20	20	0	
Douglas	20	20	0	
Ferry	5	3	**1**	1 larva
Garfield	20	3	**1**	1 adult
Gray's Harbor	5	5	0	
Island	5	5	0	
Jefferson	5	5	0	
King	20	10	0	
Kitsap	5	5	0	
Kittitas	5	6	0	
Klickitat	5	9	0	
Lewis	20	11	**1**	1 larva
Mason	5	5	0	
Okanogan	5	5	0	
Pacific	5	5	0	
Pierce	20	20	0	
San Juan	5	5	0	
Skagit	20	20	0	
Skamania	5	5	0	
Snohomish	20	12	0	
Thurston	20	10	0	
Walla Walla	20	11	0	
Wahkiakum	5	5	0	
Whatcom	20	20	0	
Whitman	20	22	**1**	1 adult
Yakima	5	5	0	
Total Surveyed 2002		275	5	

Bold indicates high-priority county

All 30 prioritized counties were surveyed according to standardized methods. The number of sites surveyed in each county varied depending on the pre-assigned priority status. A minimum of five sites per county was satisfied in all counties, unless the county was declared positive. In the high-priority counties of Benton, King, Lewis, Snohomish, Thurston, and Walla Walla, the target number of sites (20) was not achieved due to limited sampling time and resources.

Host plants surveyed in 2002 included cultivated grains and grasses grown for food, seed or forage. Specimens from four of the five positive sites were collected directly from cultivated oat or barley fields (Table 2). Although the Whitman County specimen was trapped in a wooded site, Lyle Grove is typically surrounded by several contiguous acres of cultivated wheat.

Table 2: Host Plants Surveyed, 2002

Host Plant	Total # Sites Surveyed	# Positive Sites
Oats	28	2
Wheat	105	1
Barley	15	2
Rye	6	0
Forage Grasses	119	0
Corn	1	0

Forage grasses sampled were typically grown for hay, pasturage, and silage, and composed of many species of Gramineae, including timothy, orchard grass, brome, wild oats, reed canary grass, sorghum, Kentucky bluegrass, ryegrass, and Sudan grass. Detections in 2002 were made in both irrigated and non-irrigated grain fields.

2. Delimiting Survey

Delimitation survey began on May 14. Limited sampling resources prevented a systematic delimitation of the geographic range of CLB in positive counties. When time allowed, known positive sites were revisited to confirm the positive status (Table 3). Sites in Clark, Lincoln and Spokane counties were reconfirmed as positive in 2002. Six new positive sites were also reported and confirmed in known infested counties.

Table 3: CLB Delimitation Survey, 2002

County	Total # Sites Sampled	# Old Sites Revisited 2002	# Old Sites Reconfirmed 2002	# New Positive Sites	Total # Positive Sites, All Years (1999-2002)
Adams	0	0	0	**0**	**2**
Clark	3	1	1	**0**	**3**
Columbia	1	0	0	**0**	**1**
Franklin	0	0	0	**1**	**2**
Grant	2	0	0	**2**	**4**
Lincoln	3	1	1	**0**	**3**
Pend Oreille	0	0	0	**0**	**2**
Spokane	5	3	1	**2**	**11**
Stevens	1	0	0	**1**	**6**
TOTAL	**15**	**5**	**3**	**6**	**31**

In Spokane County, CLB was found at two new sites in the Peone Prairie region. One site showed significant feeding damage, prompting the grower to initiate chemical treatment of 490 acres of

spring wheat. This was the first report in Washington State of chemical application for control of CLB.

Several late-season reports from farmers and researchers identified heavy infestations in Stevens and Franklin counties. In Stevens County, "heavy" numbers of CLB were reported in wheat, oats and barley fields north of Colville. The populations were dense enough to trigger a chemical application of 225 acres for control of the beetle. Separate reports also indicated heavy CLB populations in research plots in northern Franklin County and Garfield County. Although quantification of these populations was not possible in 2002, future surveys will monitor the densities and damage caused by the insect.

Aerial application for control of CLB population in spring wheat (Spokane, Washington, 2002).

When time allowed, known CLB-infested areas were visited to monitor the population levels. Five positive sites were sampled to estimate population density. Table 4 lists the estimates for these sites. The Brush Prairie site still remains below measurable levels. Comparison of density from 2002 at the Nine Mile Falls site showed an increase in population from 1.05 CLB/tiller (July 29, 2001) to 2.12 CLB/tiller (July 11, 2002). Due to variability in sampling dates and methods, it is not possible to compare data between 2000 and 2002. An ongoing seasonal study of CLB phenology will provide the necessary data for a clearer comparison between years.

Table 4: CLB Population Levels at Selected Sites

County, Site	2000 Population (Avg # CLB/Tiller)[1]	2001 Population (Avg # CLB/Tiller)[2]	2002 Population (Avg # CLB/Tiller)[2]
Clark, Brush Prairie	-	NA	**0**
Grant, Stratford	-	NA	**0.13**
Spokane, Nine Mile Falls	**0.37**	**1.05**	**2.12**
Spokane, Peone 1	**0.67**	-	**0.53**
Spokane, Peone 2	-	-	**1.26**

[1] N= 30 tiller [2] N= 100 tiller

3. ***Biocontrol***

Pre-release Survey

Prior to the 2002 release of bioagents, 300 larvae were hand collected from the Nine Mile Falls field insectary by WSDA and USDA-Niles staff. Larvae were returned to the WSDA lab and USDA-Niles Lab and dissected to monitor for *T. julis* parasitism as evidence of establishment. No parasitism was observed during this second-year evaluation.

Augmentation

Two separate shipments of parasitized CLB larvae were collected from insectaries in Montana and Pennsylvania (see Table 5). Parasitized CLB larvae were swept and hand-collected from a field insectary in Ronan, Montana during the 2002 CLB Workshop/Bootcamp. Dissections estimated parasitism rates for each collection site, ranging from 67% to 100% parasitism. Parasitism rates also demonstrated gregarious qualities, ranging from 0 to 8 parasites per host, and averaging 4.9 *T.julis* per CLB (N=20).

Table 5: *Tetrastichus Julis* Introductions to Nine Mile Falls Insectary in 2002				
Date Released	**Origin**	**# CLB Larvae Released**	**% Parasitism**	**Estimated # T. Julis***
June 14	Pennsylvania	2,225	100%	11,125
June 28	Montana	4,253	80,70,60%	21,263
TOTAL	-	6,478	-	32,388

* Estimate based on an average of 5 *T. julis* per 1 CLB larva.

All shipments of *T. julis* were released at the Nine Mile Falls insectary site. The June 14 shipment was released in fields with previously defined "conservation strip" areas. The June 28 shipment was released into a new managed field insectary plot.

For 2003, startup funds have been provided by the USDA-APHIS-western region to reinforce biocontrol efforts in Washington State. Through collaborative efforts, two local farmers have committed acreage to managing insectary sites for the production of two species of biological control agents. Using a contiguous-plot design, the insectaries will be managed for the next five years to support a CLB population and provide protection for overwintering parasitoids. The Nine Mile Falls insectary site, located in Spokane County, will serve as the *T. julis* field insectary (Nine Mile Falls) and a second insectary site (west Peone Prairie) will provide management for the production of the egg parasitoid *A. flavipes.* During 2002, no releases of *A. flavipes* were made; however, winter grains were seeded in preparation for the first releases of the parasitoid planned for spring of 2003.

Five Tetrastichus julis parasitoid larvae from a dissected CLB larva. Collected from field insectary in Ronan, MT.

REGULATORY ISSUES

WSDA and USDA support growers and shippers of commodities, such as small grains, hay and straw, in their attempt to meet domestic (Californian) and foreign (Canadian) quarantine requirements. Through annual survey results and certification programs, WSDA can declare certain Washington counties as "CLB-free" areas. Under WSDA certification, commodities are allowed entry into foreign markets without undergoing phytosanitary treatment (i.e., fumigation). Existing quarantines imposed by Canada were enforced in 2002, with substantial effects on Washington's hay industry. In the fall of 2002, the USDA-APHIS Spokane office supervised 48 tarpaulin bulk stack fumigations of hay and straw (alfalfa, wheat straw and bluegrass), totaling approximately 5,000 tons shipped to Canadian markets. One company estimated a $50,000 cost to the industry for its tarped hay fumigations from September to November.

The California quarantine also hindered shipments of certain Washington commodities in 2002. Regulated commodities such as grass hay, straw, small grains, and seed corn (ear corn) experienced regulatory obstacles, increased costs, and even loss of buyers in California. As the geographic range of CLB expands in major hay and grain producing regions of Washington State, additional counties will most likely be affected by these foreign and domestic quarantines.

CONCLUSION

As of 2002, a standardized detection survey has been conducted in all 39 Washington counties. Five new positive county records were confirmed in 2002: Asotin, Ferry, Garfield, Lewis and Whitman. For regulatory purposes, positive records of cereal leaf beetle in Washington now exist for fourteen counties, primarily occurring in the eastern area of the state. Within CLB-positive counties, delimitation surveys reconfirmed three historical sites and identified six new positive sites. No parasitoids of CLB have been recovered or detected in Washington State. Supplemental releases of the biological control agent *Tetrastichus julis* are planned for the Nine Mile Falls field insectary. Initial releases of the egg parasitoid *Anaphes flavipes* are planned for 2003 at the Peone Prairie field insectary.

While most known CLB populations exist well below economic threshold levels, several accounts of heavy infestations were reported in 2002. Large CLB populations in northern Spokane and Stevens counties prompted pesticide treatments of 715 acres of wheat, oats and barley, signifying the first chemical applications for control of CLB in Washington State. The insect will most likely continue to expand its range, exposing Washington industries to the requirements of Canadian and Californian quarantine regulations. Without the presence of natural enemies, rising CLB populations will intensify the need for chemical control in the grain-growing regions of Washington State.

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SUMMARY of ACTIVITY

Detection and Delimitation Surveys (See CLB detection survey map)

- Standardized survey completed in all 39 Washington counties: results = 14 positive counties.
- Current positive counties reported (with total number of positive sites):
  Adams (2), Asotin (1), Clark (3), Columbia (1), Ferry (1), Franklin (2), Garfield (1), Grant (4), Lewis (1), Lincoln (3), Pend Oreille (2), Spokane (11), Stevens (6), Whitman (1).
- Sites in Clark, Lincoln and Spokane counties were reconfirmed positive in 2002. New populations were identified in Franklin, Grant, and Stevens counties.
- Significant populations identified near Colville (Stevens County), Stratford (Grant County), Connell (Franklin County), and Nine Mile Falls and Peone Prairie (Spokane County).
- First reports of chemical application for control of CLB in Washington State. Approximately 225 acres north of Colville (Stevens County), and 470 acres in the Peone Prairie (Spokane County).

Biological Control

Survey Summary	1999	2000	2001	2002
Duration	June 3 – July 28	Apr 27 – July 28	Apr 25 – Aug 30	May 1 – Aug 14
Total Counties Surveyed	9	16	23	30
Total Sites Surveyed	135	200	186	290
New Positive Counties	1	3	5	5
Total Positive Counties	1	4	9	14
New Positive Sites, Detection	4	8	9	5
New Positive Sites, Delimitation	-	4	3	6
Total Positive Sites	4	16	28	39

- Pre-release evaluation study resulted in no second year recovery of the larval parasitoid *T. julis* in 2002.
- Release of approximately 6,500 parasitized CLB larvae at field insectary in 2002. Based on % parasitism, an estimated 32,000 *T. julis* larvae were introduced. Total estimated number of *T. julis* introduced to insectary for three years: 42,000 larvae or adults.
- Further support to encourage establishment of *Tetrastichus julis* at the Nine Mile Falls insectary, through designation of five acres of cultivated land and management strategy, field insectary plot.
- Support for establishment of a second field insectary site for the propagation of egg parasitoid *Anaphes flavipes*. Management plan and first planting begun.

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CLB 2002 Detection Survey

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